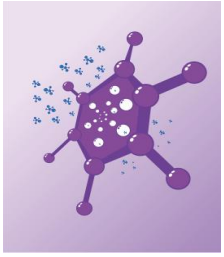
Journal of Membranes and Materials v(i) pp-pp (YYYY)
Received Month Year / Revised Month Year / Accepted Month Year

Journal of Membranes and Materials

https://ejournal2.undip.ac.id/index.php/jmm



Separation of Saponin Using Nanofiltration Membrane

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Abstract. Separation of pure saponin and saponin-BSA protein mixture by nanofiltration membranes have been investigated in this study to understand the nanofiltration potential to obtain high purity saponin. Commercial NF membranes: NF, NF270, and DSS-ETNA01PP were used. The effects of the operating conditions such as pressure, the concentration of feed, and the composition of feed were evaluated. The permeate flux and rejection rate of saponin and saponin-BSA were the criteria of this evaluation. The increasing operating pressure increased the permeate flux. In addition to the membranes' MWCO, electrostatic repulsion between the charged membrane interface and solute determined the saponin and saponin-BSA solution's rejection rate. The flux of pure saponin feed was greater but generated lower rejection rates than the saponin-BSA feed. Increasing feed concentration resulted in an increased rejection rate. However, the flux decreased with increasing pure saponin concentration but increased with a higher dose of saponin-BSA. The DSS-ETNA01PP membrane had the largest flux value and the smallest rejection value compared to other membranes. The results indicated that nanofiltration was potential for the saponin purifying process.

Keywords: Electrostatic; Nanofiltration; Rejection; Repulsion

1. Introduction

Saponins are secondary metabolic products found in plants with high molecular weight. Saponins can be found in dicot and monocot plants, including *Camellia sinensis*, *Aesculus hippocastanum*, *Rosa centifolia*, *Swietenia mahogany*. Saponins function as chemical barriers or protectors in plant self-defense systems against pathogenic bacteria and herbivores (Augustin et al., 2011). Saponins are composed of sugar units linked to triterpene or steroid aglycones. Saponins generally have detergent-like properties, reducing the surface tension in aqueous solutions and forming a stable foam. Saponins can dissolve in various solvents such as water, ethanol, and methanol. It is partly soluble in ether, chloroform, benzene, ethyl acetate, or acetic acid (Hostettmann and Marston, 1995).

Saponins are widely used in the cosmetics, agriculture, food, and pharmaceutical industry. They have hemolytic, anti-inflammatory, anti-yeast, antimicrobial, antiparasitic, anti-tumor, and antiviral properties (Sparg et al., 2004). The discovery of saponins' biological activity triggered the semi-synthesis of steroid drugs in the pharmaceutical industry.

With the increasing use of saponins, many studies have been conducted to obtain commercial-scale saponins from plants (Guclu-Untundag and Mazza, 2007). The most used attempt is by carrying out extraction, which several methods can do, including maceration

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doi: 10.14716/ijtech.v0i0.0000

(Takeuchi et al., 2009), reflux and soxhlet (Bart, 2011), ultrasonic (Wu et al., 2001), and microwave (Vongsangnak, 2004).

However, the extracted saponin content is still insufficient, so further purification steps are needed to obtain high saponin content (Guclu-Untundag and Mazza, 2007). The saponin purification process can be conducted in several ways, including solvent precipitation (Kitagawa, 1986; Nozomi et al., 1986), adsorption (Giichi, 1987), and chromatography (Kensil and Marciani, 1991). Chromatography is often used in laboratory-scale saponin purification processes such as open column chromatography, thin-layer chromatography (TLC), liquid chromatography, and countercurrent chromatography (Hostettmann and Marston, 1995). However, commercial-scale saponin production using this method is not economical (Guclu-Untundag and Mazza, 2007).

Another method that can be applied for saponin purification is nanofiltration. This technology does not require additional chemicals, operates isothermally at room temperature, and consumes low energy (Susanto, 2009). Nanofiltration membranes procure very high rejections for multivalent ions (>99%), low to moderate rejections for monovalent ions (0–70%), and high rejection (>90%) for organic compounds with a molecular weight above the membrane's (Norman et al., 2008).

This study aimed to discover the potential of nanofiltration membranes for obtaining high purity saponins. The membranes' performance and characteristics would be assessed for the process with various membrane types, pressures, feed compositions, and feed concentrations.

2. Methods

2.1. Materials

The materials used in this study were saponins (Sigma Aldrich, 8-25%), BSA protein (Sigma Aldrich, ≥98%), vanillin (Sigma Aldrich, 99%), H_2SO_4 (Sigma Aldrich, 96 %). The membranes used were NF (Alfa Laval), NF270 (FILMTEC), and DSS-ETNA01PP (Alva Laval).

2.2. Flux Measurement

The membrane was cut with a diameter of 4.2 cm, then soaked for 30 minutes in distilled water. The membrane was inserted into the membrane module and compacted for 30 minutes with pressure above the operating pressure (5, 6, 7 bar). The feed was filled with distilled water. The distilled water flowed through the filtration unit for 15 minutes at operating pressure (4, 5, 6 bar) to obtain J_0. Afterward, the permeate was collected and weighed. Then, the saponin feeds (pure/mixed with BSA) were put in the feed tank, filtered for 2 hours at specific operating pressures. The permeate was collected and weighed every 15 minutes to measure the flux.

2.3. Rejection Analysis

The rejection analysis was done spectrophotometrically. The water was heated to 60°C. Five ml of 72% H_2SO_4 solution was put in a container covered with aluminum foil. Vanillin solution of 8% w/v was made. Half ml of it was put in the container containing H_2SO_4 solution and rested for 1 minute. Half ml of permeate from the filtration process was added into the container containing vanillin and H_2SO_4 mixture and rested for a minute. The container was then heated for 10 minutes in hot water and cooled in the ice water for 5 minutes. The mixture's absorbance was measured using a spectrophotometer (Genesys 20) at the wavelength of 544 nm.

3. Results and Discussion

3.1. Effect of Pressure on Flux in NF270 and DSS-ETNA01PP Membrane

The filtration process of pure saponin and saponin-BSA protein with operating pressures of 4, 5, and 6 bar using NF270 and DSS-ETNA01PP membranes was done to investigate the effect of pressure on flux. The feed concentrations were varied from 50, 100, to 150 ppm. The results can be seen in Figure 1.

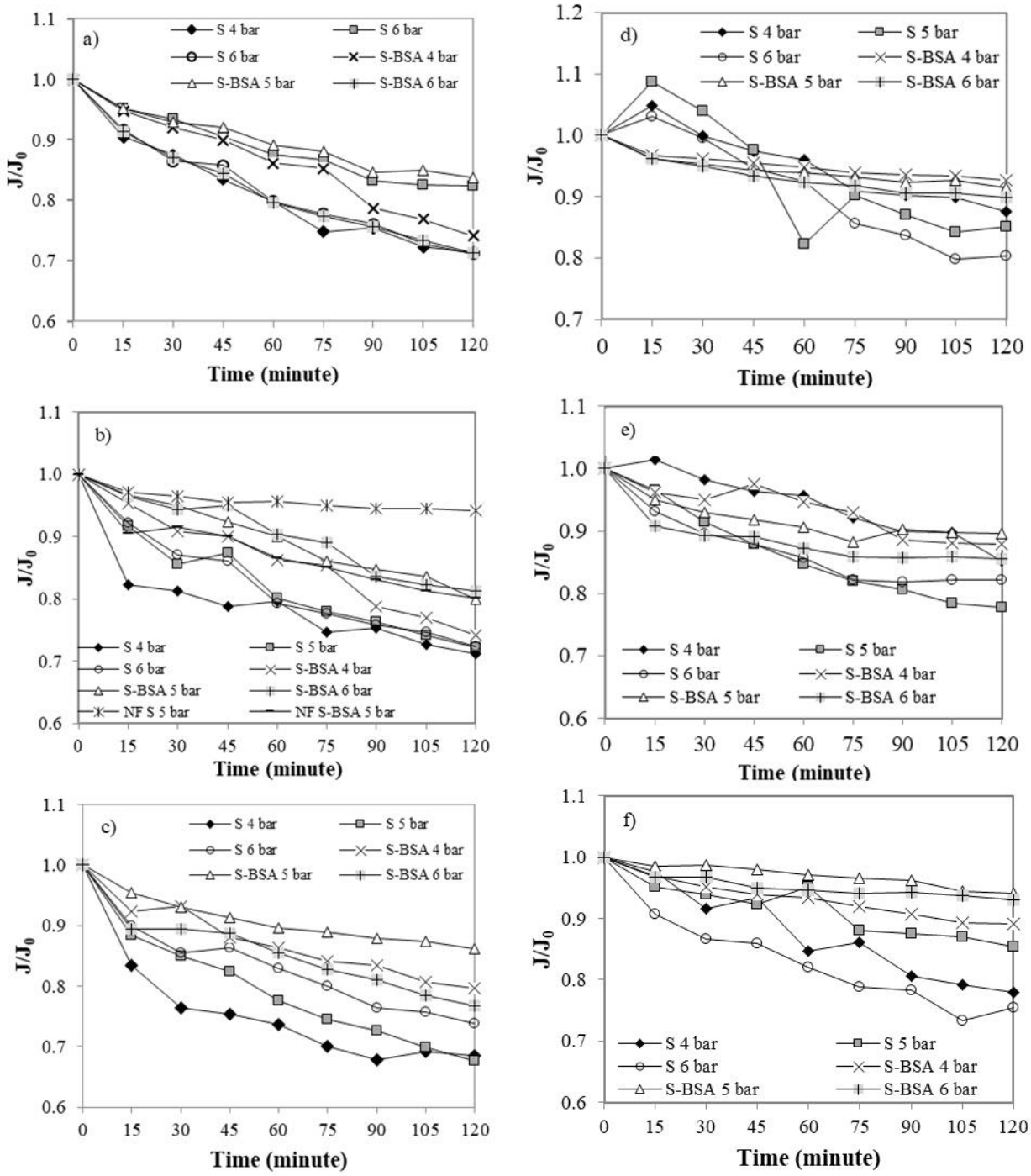

Figure 1 Flux profiles of various feed concentrations: a) NF270-50 ppm, b) NF270-100 ppm, c) NF270-150 ppm, d) DSS-ETNA01PP-50 ppm, e) DSS-ETNA01PP-100 ppm, f) DSS-ETNA01PP-150 ppm

Figure 1 shows that the flux profile decreased with a longer operating time on both membrane types. The flux drop was relatively consistent with each pressure variation. For NF270, the flux of 5 bar had the most optimal value than of 4 and 6 bar at 50 ppm. On the other hand, at the concentration of 100 ppm and 150 ppm, there was no significant difference in each pressure variation's flux.

The pressure of 4 bar resulted in the smallest flux; this was because the crossflow's driving force was less significant. So that the molecules accumulated on the membrane surface were not swept away by the recycle flow.

The flux reduction using the DSS-ETNA01PP membrane was relatively consistent at each pressure variation. As the filtration operation time increased, the resulting flux also decreased and became more stable at the end of the operation time. This phenomenon occurred both in pure saponin and saponin-BSA feed.

Mixed feed solution of saponin and BSA showed increased flux value with increasing operating pressure. At a pressure of 6 bar, the resulting flux was higher compared to other operating pressures because the driving force applied was more significant so that more solutions could pass through the membrane.

According to Lin et al. (2004), the decrease in normalized flux occurred due to fouling and polarization concentration on the membrane surface. Besides, protein molecules' nature is easily adsorbed by membrane surfaces and pores, making BSA a foulant that is quite difficult to control (Wei et al., 2006). The longer the operating time, the more BSA would be deposited on the membrane's surface and pores. It resulted in the flux decrease.

As the operating time increased, the resulting flux decreased, while at the end of the operating time, the flux value became more stable. This phenomenon was caused by fouling and polarization concentration on the membrane surface. Fouling is the deposition of suspended substances, usually solutes, which results in decreased membrane performance and is irreversible. Meanwhile, polarization concentration occurred due to solute accumulation that stuck on the membrane surface, so that it caused flux decrease and is reversible (Lin et al., 2004; Sutzkover-Gutman et al., 2010).

In this saponin filtration, fouling occurred because of the sieving mechanism—the molecule size difference between the solute molecules and the membrane pore size caused the separation process. Saponin compounds have a molecular weight of 414.63 Da, and BSA has a molecular weight of 66,430 Da. Meanwhile, the pore size or Molecular Weight Cut Off (MWCO) of the NF-270 membrane is 180 Dalton. Theoretically, saponin compounds and BSA protein compounds would be stuck on the membrane surface because they had a larger molecular size than the membrane pores. As the filtration operation time increased, more molecules would cause fouling on the membrane, resulting in the flux decreasing.

3.2. Effect of Pressure, Feed Composition, and Feed Concentration on Rejection Rate

Pure saponin and saponin-BSA protein filtration processes using NF, NF270, and DSS-ETNA01PP were done to investigate the effect of pressure on rejection rate. The operating pressures were 4, 5, and 6 bar while the feed concentrations were 50, 100, and 150 ppm. The results are presented in Table 1.

The pure saponin solution feed that passed on the NF270 membrane showed that the higher the feed concentration, the greater the rejection rate. Pedebos et al. (2014) reported that carboxyl groups in saponin made the feed solution negatively charged. The NF membrane's surface has been known to be negatively charged. As the concentration of the solution increased, the number of saponin molecules would also increase. It resulted in greater repulsion force (electrostatic repulsion) between the membrane surface and the solution. Therefore, the higher saponin feed concentration increased the rejection rate of the NF270 membrane.

In the saponin-BSA mixed feed, the rejection data show an increase in the rejection rate from 50 ppm to 100 ppm then slightly decreased at a concentration of 150 ppm. The increase in rejection rate was caused by electrostatic repulsion from the membrane surface and solute interaction. In a study conducted by Chaiyasut and Tsuda (2001), the BSA molecule had an isoelectric point at pH 4.6-4.7. It is a condition where the BSA molecule's net charge is zero (Salgin et al., 2012). In this study, the saponin-BSA mixed feed solution pH was above 5, indicating that the BSA molecule was negatively charged. Therefore, the electrostatic repulsion became more significant with increasing feed concentration, resulting in a higher rejection rate.

Table 1 Rejection Rate of the Membranes

Membrane	Feed	Concentration	% Rejection		
			4 bar	5 bar	6 bar
NF270	Pure Saponin	50 ppm	23.3	21.6	24.4
		100 ppm	35.9	30	31.1
		150 ppm	56.8	55	43
	Saponin - BSA	50 ppm	43.8	41	40
		100 ppm	74	70.5	67
		150 ppm	73.7	68.7	62.5
NF	Pure Saponin	100 ppm	N/A	55.5	N/A
	Saponin - BSA		N/A	63.4	N/A
DSS-ETNA01PP	Pure Saponin	50 ppm	40.7	57.1	60.5
		100 ppm	31.7	60.5	71.6
		150 ppm	33.7	41.2	53
	Saponin - BSA	50 ppm	33.8	35.3	29.4
		100 ppm	52	48.1	29.4
		150 ppm	52	63.9	59.8

At the same operating condition, the rejection rate of the saponin-BSA mixture feed was greater than the pure saponin feed. Carvalho et al. (2011) reported that the membrane and ionic charges in the solution provided additional rejection because of the electric and dielectric effects. Thus, apart from the sieving mechanism effect, saponin separation on the NF membrane also occurred through an electrostatic repulsion mechanism. The NF membrane's surface and the pure saponin solution were negatively charged, inducing repulsive force.

Saponin-BSA mixture was more negatively charged than the pure saponin, creating greater electrostatic repulsion that generated a higher rejection rate and increasing feed concentration.

The rejection data of filtration using DSS-ETNA01PP membrane in Table 1 shows a decrease in rejection rate with increasing pure saponin solution feed concentration. Meanwhile, the saponin-BSA mixture filtration's rejection rate had the opposite phenomenon with the pure saponin feed. As the mixed feed concentration increased, the resulting rejection also increased. It was because the saponin-BSA mixed solution had different properties than the pure saponin. Kezwon and Wojciehjowski (2014), in their research on saponin-protein interactions in food, concluded that saponins would aggregate with protein molecules due to the saponin properties, which could reduce surface tension and also had a high aggregation behavior. Based on these properties, the higher the solute concentration in the feed solution, the more molecules would form the aggregates resulting in a wider molecular diameter. With a wider molecular diameter, theoretically, it could not pass through the smaller membrane pores. Therefore, the rejection rate would increase as the concentration of the saponin-BSA mixture feed increased.

3.3. Characterization of membrane fouling

SEM analysis is one way to characterize membrane fouling from the membrane surface and membrane pore cross-sections. The SEM test results of the three membranes used to filter pure saponin solution and saponin-BSA protein solution are presented in Figure 2.

According to Figure 2, there was no significant difference seen in both the membrane used to filter pure saponin and saponin-BSA protein. There was fouling on both used membranes indicated by oval-shaped molecules, which belonged to saponins. In the membrane used to filter saponin-BSA protein solution, round molecules were seen, representing the BSA protein. If we

look at the cross-section SEM analysis results, the used DSS-ETNA01PP membranes had the largest pores shaped like fingers.

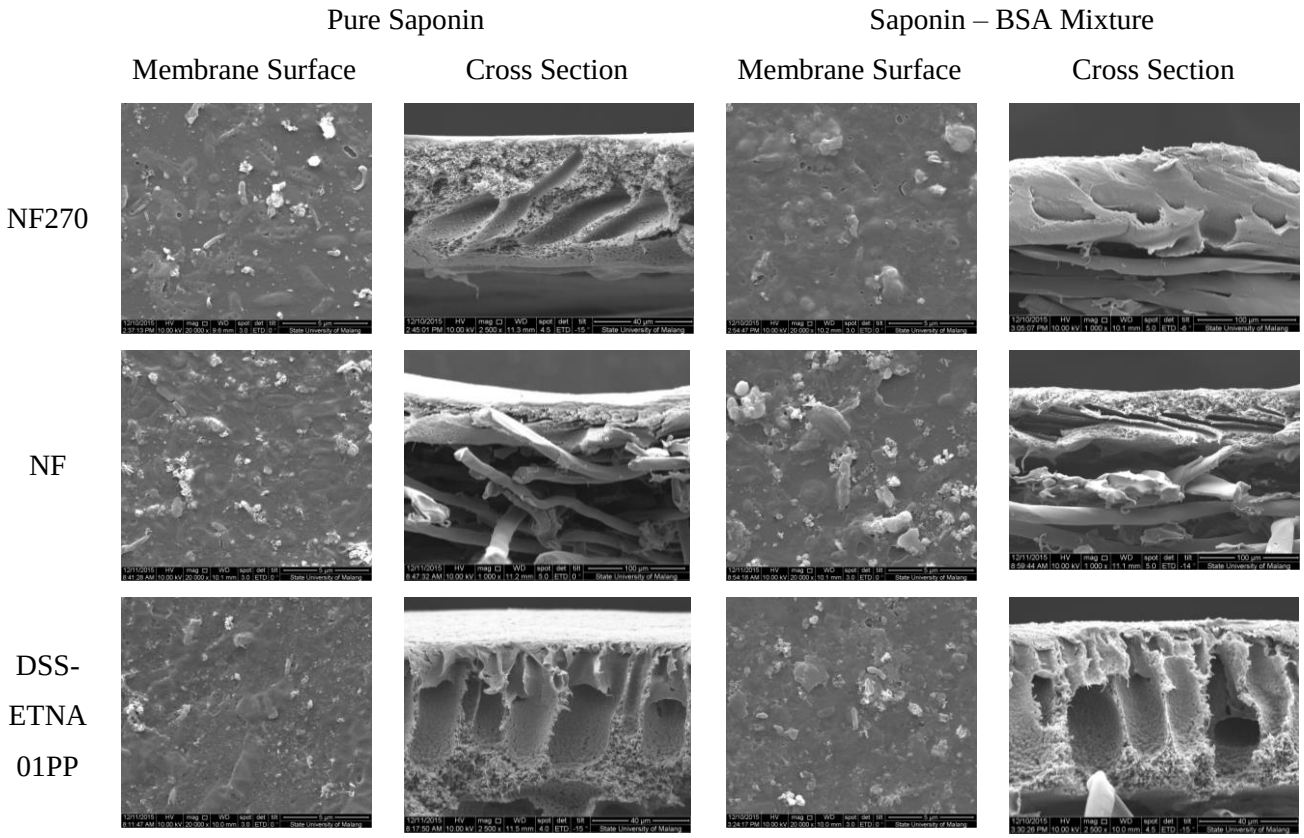

Figure 2 SEM characterization of the membranes used to filter 100 ppm of pure saponin and saponin-BSA protein

4. Conclusions

This research aimed to know the potential of purifying saponins using a nanofiltration membrane. Increasing operating pressure caused the flux to increase and the decreased rejection value. The flux of pure saponin feed was greater but generated lower rejection rates than the saponin-BSA feed.

Increasing feed concentration resulted in an increased rejection rate. However, the flux decreased with increasing pure saponin concentration but increased with a higher dose of saponin-BSA. The DSS-ETNA01PP membrane had the largest flux value and the smallest rejection value compared to other membranes. The results indicated that nanofiltration was potential for the saponin purifying process.

Acknowledgements

The authors would like to thank the Membrane Research Center (MeR-C), Integrated Laboratory, Diponegoro University for funding this research.

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